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Award Number: DAMD17-98-1-8580

TITLE: Chemoprevention Trial of Selenium and Prostate Cancer

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REPORT DATE: April 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 2003	Annual (1 Apr 02 - 31 Mar 03)

4. TITLE AND SUBTITLE
Chemoprevention Trial of Selenium and Prostate Cancer

5. FUNDING NUMBERS
DAMD17-98-1-8580

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

The principal purpose of this trial is to assess the potential for the essential nutrient **selenium** (Se) to modulate biomarkers of prostate cancer between initial diagnostic biopsy and radical prostatectomy. The scope of work is to randomize at least 110 participants to either a placebo or Se dosages of 200 μg, or 400 μg/day. Recruitment is continuing as a result of a no-cost extension. A total of 79 subjects have been randomized. Of these, 72 have completed the study, 6 dropped before completing the study, and 1 is in the process of completing the study.

14. SUBJECT TERMS:
prostate cancer, selenium, prostatectomy, biopsy, randomize, placebo

15. NUMBER OF PAGES
9

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

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Frederick R Ahrens 4/28/03

PI - Signature **Date**

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INTRODUCTION

The principal purpose of this trial is to assess the potential for the essential nutrient selenium (Se) to modulate biomarkers of prostate cancer. The rationale for this trial is based on the results of the Nutritional Prevention of Cancer (NPC) Trial. In that study, a double-blind, randomized clinical trial, a 63% reduction in prostate cancer incidence was observed during the initial 10 years of follow-up in participants receiving 200 μg of Se compared to those receiving a placebo (JAMA 276:1957-63 (1996)). Objective: The primary endpoint for this trial consists of changes in biomarkers between tissues obtained at the initial diagnostic biopsy and radical prostatectomy. Relevance: This study has the potential to provide direct evidence for the activity of selenium in prostate tissue. Methods: A study population of prostate cancer subjects scheduled for prostatecomy was selected so that prostate tissue can be examined for biomarker changes before and after supplementation with selenium. This trial will randomize participants to either a placebo or one of two Se dosages: 200 μg, or 400 μg/day. The trial will randomize at least 110 patients, in order to have 80% power to detect an effect size of 0.66 standard deviations. Progress: A total of 79 subjects have been randomized. Of these, 72 have completed the study, 6 dropped before completing the study, and 1 is in the process of completing the study.

PROGRESS

Task 1: Training and Preparation for Trial (Months 1-36 - Ongoing)

- A database has been created for this study and staff at the Tucson Coordinating Center (TCC) have been trained in its use. Routine reports are available to assist staff in tracking subjects from initial referral through randomization.
- Staff at TCC and study sites have been trained to explain the study requirements to subjects and to inquire about adverse effects. TCC laboratory staff have established routines to ensure that the proper blood kits for the various tests performed after each visit are used.
- Randomization codes have been prepared and appropriate staff have been blinded to blood tests results that might reveal the subject's treatment.
- Pills are dispensed according to randomization codes by staff blinded to treatment status.
- An "Initial Questionnaire", "Follow-up Study Visit" questionnaire, and "Urological Symptoms Questionnaire" have been developed. A food frequency questionnaire developed by the Fred Hutchinson Cancer Center in 1992 is also being administered to study subjects.
- All appropriate laboratory materials to obtain, handle, store, and prepare blood and tissue samples for analyses have been obtained.
- Training has been ongoing as new sites are added to the study.

Task 2: Subject Recruitment, Enrollment (Months 3-34- Ongoing***)***

Recruitment for this study has been slow despite frequent contact with physician offices. It now appears that participating physicians overestimated the number of eligible patients they can provide. In addition, we initially imposed a recruitment requirement for frozen tissue that led many urologists to withdraw from the study. Since we eliminated this requirement, we have been able to reestablish participation from many of the urologists who originally agreed to refer patients. Still, actual referrals have been far lower than original estimates. Factors which have contributed to the slow pace of recruitment include:

- *Time for Patient Recruitment*. The window of opportunity for enrolling subjects to this study – the three to six week period between diagnosis and surgery – limits the type of

recruitment methods available. These subjects must be identified as soon as possible after diagnosis during a time when they are struggling with the emotional impact of their diagnosis. Advertisements and health fairs, which have yielded some subjects for our other selenium and prostate cancer studies, have been ineffective for this study.

- *Inadequate Number of Referring Physicians.* During the early stages of the study, the primary focus was on Tucson urologists. Dr. Bruce Dalkin, the Co-Principal Investigator, has been the greatest source of subjects for this study. Previously, urologists at remote sites: Dr. Martha Terris at the Palo Alto VA in Palo Alto, CA, and Dr. Christopher Julian at the Urological Associates of Central California in Fresno, CA have enrolled patients on this study. In addition, the University of Arizona Cancer Center has recently established a clinic in Scottsdale overseen by Dr. Michael Gordon and subjects are now being recruited from the entire Phoenix area.
- *Protocol Changes.* Protocol changes were made in March and July 2000. The first change eliminated the requirement for frozen tissue samples and appears to have had a positive effect on recruitment. The protocol changes made in July 2000 significantly slowed the rate of physician referrals due to delays in securing IRB approval for these complex changes. These changes eliminated the follow-up portion of this study and made the changes in tissue biomarkers the primary endpoint. These changes have been approved by HSRRB.
- *Documentation requested by HSRRB.* Due to the various IRB submissions and due to delays including events related to September 11, we were not allowed to open new sites which would have accelerated recruitment. Approvals have now been granted by HSRRB for all participating sites.

Of the 79 randomized subjects, there are 2 Hispanics, 2 African American, 1 Asian, 70 Caucasian, 1 other, 3 not given.

Task 3: Baseline Data Collection (Months 3-34- Ongoing)

At time of enrollment, all participants are presented with a standard set of questionnaires and forms. This set includes an informed consent form, and a baseline questionnaire that asks detailed information about previous and current illnesses, medications (including OTC and herbal supplements or vitamins), family history of cancer, and lifestyle. In addition, dietary information is gathered using a well validated Food Frequency Questionnaire. The TCC collects biopsy tissue, medical records, a registration form, and a blood sample.

The following table summarizes data collected to-date:

Data Type	
Baseline questionnaire	78
Follow-up Questionnaire	111
FFQ	73
Blood sample	170
Urological Symptoms Questionnaire	50*
Pathology Reports	115
Frozen tissue sample	38

*Discontinued under revised protocol

Task 4: Randomization (Months 4-34- Ongoing)
There is no run-in period for this study. Subjects are randomized at the time of enrollment. Due to the short time subjects are required to participate in the study, randomization of new patients will continue throughout the study period.

Task 5: Follow-Up (Months 4-36- Ongoing)
Although the original statement of work calls for selenium supplementation and follow-up through the end of the grant period, we have limited supplementation and follow-up to the completion of prostate surgery in accordance with the revised study objectives. Under the revised study design, participants have their blood drawn and complete a follow-up questionnaire just prior to their prostate surgery. The follow-up questionnaire is designed to document pill compliance and possible adverse events.

Task 6: Laboratory Analyses (Months 3-30- Ongoing***)***
The following table describes the schedule for blood collection and analyses:

	Initial	Pre-Surgery
CMP	X	
Selenium	X	X
Lycopene	X	
Alpha Tocopherol (Vitamin E)	X	

We are continuing immunohistochemical tissue analysis for MIB-I, ki-67, bcl-2 and p53. Additional analyses are outlined below.

Task 7: Data Entry (Months 3-36- Ongoing***)***
All forms, questionnaires, and laboratory results are being entered into the database by the trained coordinators and laboratory assistants as they are received. Data are audited semi-annually during Quality Control reviews.

Task 8: Data Analyses and Report Writing (Months 28-36 to be initiated)
During the last months of the funding period, analyses of the collected data will be completed and reports and manuscripts for publication will be prepared and submitted. In addition to the originally proposed molecular markers (ki-67, p53 and bcl-2), additional immunohistochemical analyses including analyses for interleukin-6 (IL-6) and interleukin-6 receptor, hepsin, α-methyl-coa racemase and E-caherin, will be performed on tissue.

- IL-6, a cytokine downstream of the transcription factor nuclear factor kappa B (NFκB), has been shown to be upregulated in prostate cancer [1]. Hobisch and colleagues demonstrated that IL-6 is expressed at a low level only by basal cells in normal prostate tissue BPH. However, in prostate cancer, expression is increased and is also seen in atypical intraluminal cells.
- Immunohistochemical analyses for α-methyl-Coa Racemase (P504S) willalso be performed. P504S is an enzyme involved with metabolism of branched chain fatty acids in the peroxisome. This enzyme is overexpressed in prostate cancer tissue and some studies have suggested that the increase in expression pattern appears to correlate with Gleason grade [2].

- Expression of the adhesion molecule, E-cadherin, will also be examined. E-cadherin is involved with cell-cell interaction and expression has been shown to be lost with progression of prostate cancer [3, 4].

KEY RESEARCH ACCOMPLISHMENTS

Analyses have not been completed.

REPORTABLE OUTCOMES

Analyses have not been completed.

CONCLUSIONS

This innovative Phase II clinical trial, the *Chemoprevention Trial of Selenium and Prostate Cancer*, will provide new information on biological endpoints in a population of ethnically diverse men with localized PCa prior to the initiation of other therapy. The effect of selenium supplementation on study participants who have undergone surgical resection of the prostate will provide insight into the possible effect of selenium supplementation on biomarkers for PCa and potential mechanisms of Se action. This research can provide direct evidence for the effects of selenium on prostate tissue by examining this tissue before and after selenium supplementation.

REFERENCES –

1. Duffield-Lillico AJ, Dalkin BL, Reid ME, Turnbull BW, Combs GF Jr, Slate EH, Marshall JR for the NPC Study Group. Selenium supplementation, baseline plasma selenium status, and incidence of prostate cancer: an analysis of the complete treatment period of the NPC Trial. Submitted to BJU International, May 2002.

2. Duffield-Lillico AJ, Reid ME, Turnbull BW, Combs GF Jr, Slate EH, Fischbach LA, Marshall JR, Clark LC. Baseline characteristics and the effect of selenium supplementation on cancer risk in a randomized clinical trial: a summary report of the Nutritional Prevention of Cancer trial. Cancer Epidemiology, Biomarkers and Prevention, in press.

3. Nelson M, Reid ME, Duffield-Lillico AJ, Marshall JM. Prostate cancer and selenium. Urologic Clinics of North America, 29:1-4, 2002.

4. Clark LC, Combs GF Jr, Turnbull BW, et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group [see comments] [published erratum appears in JAMA 1997 May 21; 277(19):1520]. *JAMA*. 1996; 276:1957-63. COMMENTS: Comment in: JAMA 1996 Dec 25; 276(24):1984-5, Comment in: JAMA 1997 Mar 19;277(11):880; discussion 881, Comment in: JAMA 1997 Mar 19;277(11):880-1; discussion 881.

5. Hobisch A, Rogatsch H, Hittmair A, et al. Immunohistochemical localization of interleukin-6 and its receptor in benign, premalignant and malignant prostate tissue. J Pathol 2000; 191:239-44.

6. Yang XJ, Wu CL, Woda BA, et al. Expression of alpha-Methylacyl-CoA racemase (P504S) in atypical adenomatous hyperplasia of the prostate. Am J Surg Pathol 2002; 26:921-5.

7. Cheng L, Nagabhushan M, Pretlow TP, Amini SB, Pretlow TG. Expression of E-cadherin in primary and metastatic prostate cancer. Am J Pathol 1996; 148:1375-80.

8. Rubin MA, Mucci NR, Figurski J, Fecko A, Pienta KJ, Day ML. E-cadherin expression in prostate cancer: a broad survey using high- density tissue microarray technology. Hum Pathol 2001; 32:690-7.